Taiwan Epidemiology Bulletin

June 5 , 2012/ Vol.28 / No.11

Original Article

Monographs of Legionnaires' Disease

Message from the Editor-in-Chief

In recent years, since the progress of laboratory techniques and epidemic investigation on public health, many diseases which used to be neglected or less concerned have gradually been identified. Outbreaks originated from the same infective source could be detected. The cluster of Legionnaires' disease is an example. In view of the demand by public and medical professionals for the recognition of Legionnaires' disease, the editors of Epidemiology Bulletin therefore invited related professionals writing the monographs of Legionnaires' disease, and planning to publish two consecutive Issues in June this year (Volume 28) for readers. The contents cover an epidemiological analysis of Legionnaires' disease in Taiwan from 2007 to 2011, testing of Legionella bacteria in clinical and environmental specimens, the control strategies of Legionnaires' disease in public places, and the current situation of business sanitation self-regulation on Legionnaires' disease, etc.

An epidemiological analysis of Legionnaires' disease in Taiwan from 2007 to 2011

Chih-Ming Wang, Chia-Yu Hsu, En-Tzu Wang, Ju-Hsin Chen, Ding-Ping Liu

Second Division, Centers for Disease Control, Taiwan

Abstract

There had been 408 confirmed cases of Legionnaires' disease in Taiwan from 2007 to 2011, including 53 deaths. The incidence of confirmed cases is about 0.35 people per 100,000 population, which is significantly lower than the other countries in the world.

Regarding the age distribution of Legionnaires' disease cases, Taiwan has the same trend with other countries, with the incidence increasing with age. The elders are at a higher risk of Legionnaires' disease, and this will be a main target of disease prevention.

The monthly distribution of Legionnaires' disease cases shows that the number of cases peaks in the summer. This pattern is the same as other countries, but Taiwan’s curve isn’t as

evident as other countries. Thus, in order to prevent people from high-risk environments, which may lead to infection with Legionnaires' disease, institutions and businesses should self-regulate and engage in tasks such as cleaning and disinfecting the water supply system and cooling water tower on a regular basis.

Keywords: Legionnaires' disease, epidemiology

Introduction

Legionnaires' disease is caused by the *Legionella* bacteria mainly exist in the water. Hot water supply systems, the cooling towers of air-conditioning system and steam condensing equipment may contain this bacteria. In fact, it has been isolated from cold water, hot water and shower water, as well as their source streams, ponds and even soil. The bacteria can survive in tap water or distilled water for several months [1].

Legionnaires' disease isn’t transmitted from human to human. It is mainly caused by inhalation or choking of the mist or water containing *Legionella*.

Legionnaires' disease belongs to opportunistic infections. Although everyone can be infected, people with a poor immune system constitute a high-risk group, For example, smokers, diabetics, individuals with chronic lung disease, kidney disease or cancer, and people with a compromised immune system, especially those receiving corticosteroid treatment or having had an organ transplantation, are more susceptible to Legionnaires' disease. The severity of the disease tends to increase with age, and most of the patients are over 50 years old.

The incubation period of Legionnaires' disease ranges from 2 to 10 days, and it is usually 5-6 days. After the onset of disease, the patient will first have symptoms such as aversion to food, uncomfortable feeling, muscle pain and headache. Also, the disease will usually progress to high fever within a day (the body temperature usually goes up to 39.0-40.5 ° C), accompanied by chills, dry cough, abdominal pain and diarrhea. The patient's chest X-ray will show pulmonary interstitial and may progress to bilateral pneumonia, and this may even lead to respiratory failure. The death rate may be as high as 15%. Among patients with a weakened immune system, the death rate is even higher [2-3].

Materials and Methods

1. Case definition: Confirmed cases were required to meet both the clinical requirements and examination requirements [4].

   A. clinical requirements:

   The major symptom is pneumonia, and should accompanied by any of the following symptom: tiredness, chills, muscle pains, headache, fever, dizziness, cough, nausea, abdominal pain, diarrhea and dyspnea.

B. Lbortary requirements:

The patient should meet any one of the following conditions:

(1) Clinical specimens (sputum, respiratory secretions or pleural fluid) were isolated and Legionella spp was found in the specimens.

(2) Positive result on urine antigen screening.

(3) Positive result on serological antibody examination: If the antibody titer found during the recovery period (4 to 12 weeks) is at least four times higher than the level found during the early phase of the disease, and if the level is also equal to 128 or higher.

2. Data analysis

Using the "epidemic data storage BO (business objects) system" of the CDC, we set conditions to obtain the data on Legionnaires' disease cases confirmed during the study timeframe, and we downloaded those as EXCEL files. Also, we used records from epidemiological investigations and analyzed them to make assorted charts that we needed. In addition, epidemiological analysis was conducted to investigate correlations between the confirmed cases and their environment in this study.

Results

Descriptive statistics of confirmed cases and deaths

Based on the analysis of data from the notifiable disease reporting system of the CDC, from 2007 to 2011, the number of cases per year ranged between 50 and 100, with 81.6 cases being the annual average. Generally, the trend was a gradual increase over the years. The average male to female ratio is 3.94:1 (range from 3.00:1 to 4.78:1).

The number of deaths ranged from 7 to 14 cases per year, with the annual average being 10.2 cases. Generally, the number of deaths increased proportionally with the increase in confirmed cases over the years. Regarding mortality rates, except the higher rate (14.4%) in 2011, during the remaining four years the mortality rate of Legionnaires' disease was all about 12%.

Regarding incidence rate, it ranged from 0.24 to 0.44 people per 100,000 population over the years, with the five-year average being 0.35 people per 100,000 population, which was lower compared with the incidence in Singapore 0.65 [5], the United States 0.75 [6] and the European Union, which had an incidence of more than 1 on average (1.36,1.24,1.28 and 1.03 per 100,000 population, from 2006 to 2009, respectively) [7] (Figure 1).

Monthly incidence

According to an analysis of data from our country’s surveillance system, the monthly average of confirmed cases ranged between 4-10 cases from 2007 to 2011. In terms of the distribution of cases across the months, August had the highest number of confirmed cases, and the number generally decreased slowly after October. The curve of seasonal temperature effect

on the domestic monthly average changed more gradually and was not as noticeable as some foreign countries [6-7] (Figure 2).

Age of confirmed cases

According to the analysis of data from the notifiable infectious disease reporting system , from 2007 to 2011, the age distribution of confirmed cases indicated that no case was under 10 years old, and the incidence rate was proportional to age. It is obvious that the older the age, the higher the incidence rate of Legionnaires' disease. The incidence rate of elderly over 75 years old was as high as 2 people per 100,000 population (Figure 3).

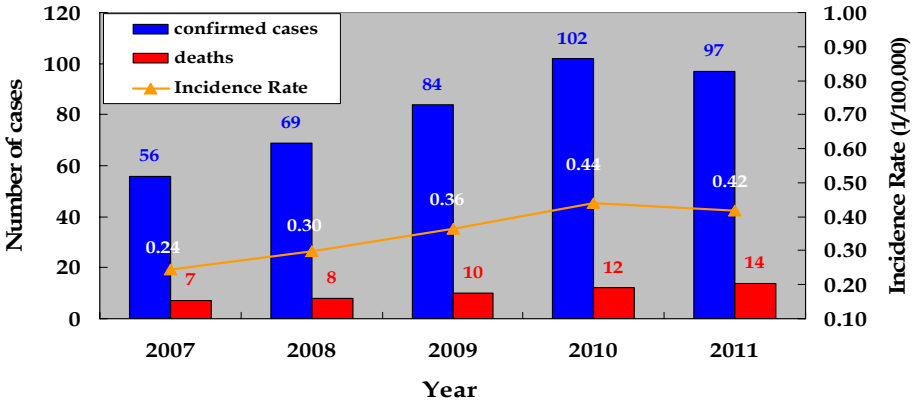

Figure 1. Legionnaires' disease in Taiwan, 2007-2011

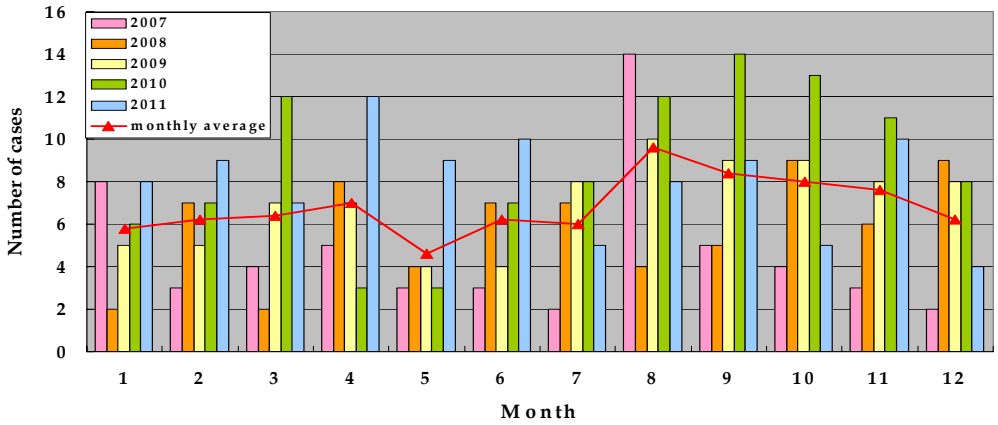

Figure 2. Monthly Cases of Legionnaires' disease , Taiwan, 2007-2011

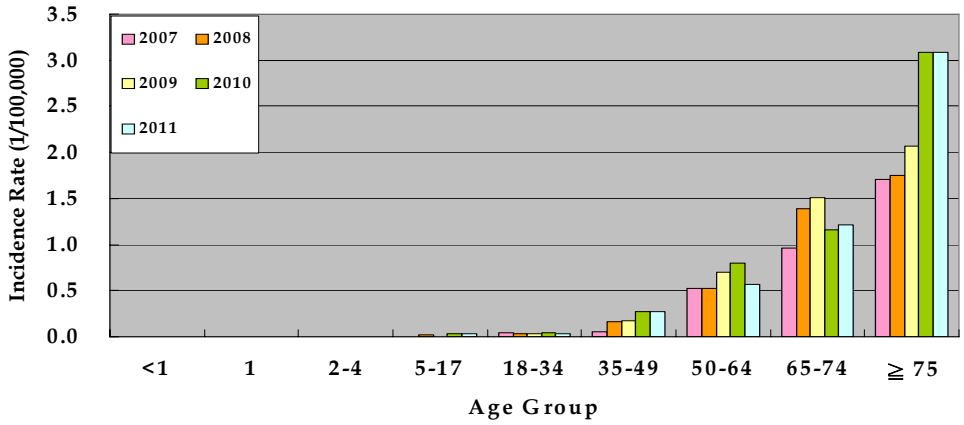

Figure 3. Incidence Rate of confirmed cases of Legionnaires' disease By Age Groups, Taiwan, 2007-2011

Age of Death

According to the analysis of data from the notifiable infectious disease reporting system, from 2007 to 2011, the age distribution of deaths showed that no case was under 30 years old. In terms of case fatality rate in different age groups, the fatality rate among young adults was relatively high, which might be due to the fact that among this group, individuals who got infected were mostly the ones with a poor immune system caused by underlying diseases. On the other hand, the fatality rate among older groups increased with age (Figure 4).

Epidemical correlation between confirmed cases and their environment.

According to data from epidemical investigations on confirmed cases, a positive epidemical correlation with the environment was found for 16 cases among the 408 cases confirmed from 2007 to 2011. Among the 16 cases (Table), 6 cases were related to the hospital, 7 cases were related to home, and 3 cases were related to commercial establishments (e.g. villa, hotel). Analysis could not be conducted on 257 of the remaining cases because there was no bacteria strain isolated from them, which was due to a lack of sputum specimens or negative sputum cultures.

Among the 16 cases, except three cases that were found by the same southern hospital in different years, no other cases were found to have any association with one other.

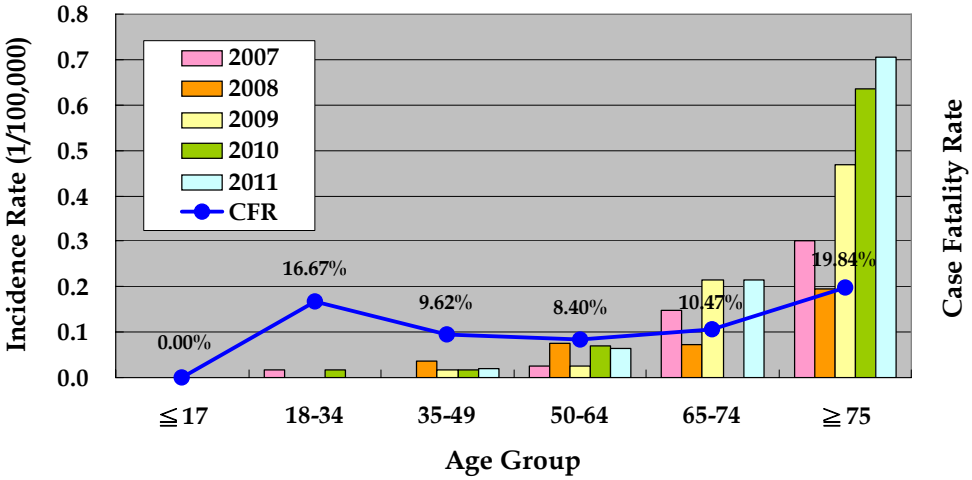

Figure 4. Incidence Rate and Case Fatality Rate of Legionnaires' disease By Age Groups, Taiwan, 2007-2011

Table Positive environments epidemically related with confirmed cases

year	Place from which positive specimens were collected（number of cases）
2007	A hospital's tap in patient's room (1), B hospital's shower head (1)
2008	A hospital's tap in the patient's room (1), drinking fountain at home (1) kitchen sink at home (1), SPA pool in villa (1)
2009	bathroom's shower head at home (1), C hospital's tap in patient's room (1), bathroom's shower head at home and face-washing water, tap in bathroom (1)
2010	A hospital's tap in patient's room (1), SPA pool in villa (1), hotel's bathtub tap (1), drinking water at home (1), drinking fountain at home (1), tap in kitchen and bathroom at home (1)
2011	D hospital's tap in patient's room (1)

Discussion

Situation of international epidemics

A. United States

According to the report released by the U.S. CDC in 2011, the total number of cases of Legionnaires' disease in the U.S.A is 22,418 people from 2000 to 2009, and the incidence rate was 0.75 people per 100,000 population. The incidence rate increased with age. Regarding incidence rates in different ethnic groups, the incidence rate was highest among Blacks and was0.87 people per 100,000 population, followed by Caucasians with an incidence of 0.59, Indians and Alaska Natives with an incidence of 0.21, and Asians with the lowest incidence of 0.14. The number of cases began to increase gradually every April and reached the peak in August and September, after which the number gradually decreased [6].

B. EU

According to the 2011 epidemiological annual report released by the European Center for Disease Control (ECDC), the incidence rate of Legionnaires' disease was about 1.03 to 1.36 people per 100,000 population. Regarding incidence rates among different countries, the rates in Italy, Spain, Denmark and France were the highest. The incidence rates in these four countries were all higher than 2 people per 100,000 population, and the incidence rate in Italy was 3 people per 100,000 population. The confirmed cases' distribution across the months showed a clear trend. The number of confirmed cases began to increase in May and reached the peak in the summer months between July and September, and then the number decreased gradually. For example, in 2009, cases occurring between July and September almost accounted for 50 percent of the total cases for that year.

It is worth mentioned that the EU puts great emphasis on the monitoring and prevention of Legionnaires' disease, particularly on the monitoring of "travel-associated Legionnaires' disease (TALD)". The "European Working Group for of Legionella Infections Network (EWGLINet)", which monitored cases of Legionnaires' disease and TALD in EU in 2009, was restructured to become the "European Legionnaires' Disease Surveillance Network (ELDSNet) " in April 2010 under the coordination of ECDC. According to data collected by this surveillance Network, in 2009, EU reported 824 TALD confirmed cases in total, and these confirmed cases belonged to 88 cluster events. These numbers from 2009 was lower than those in 2007 and 2008, which reported 947and 871 TALD confirmed cases, respectively, with the confirmed cases in 2007 and 2008 belonging to 113 and 108 new TALD cluster events, respectively [7].

Comparison of domestic and international epidemics

Observing the incidence rates of Legionnaires' disease in different countries, our incidence rate is obviously lower than those of other countries. However, in the recent five years, it has gradually increased - whether this increase was due to low levels of reporting prior to the promulgation of the "Guidelines for controlling Legionnaires' disease bacteria" in 2007 and the intensified public education effort that followed remains to be investigated. . After all, pneumonia caused by Legionnaires' disease is easily underestimated clinically. In addition, if

treatment with antibiotics preceded case reporting and specimen collection, it might affect the isolation of the bacteria. As a result, how to enhance the reporting of Legionnaires' disease is an important topic for future disease prevention efforts.

Regarding the incidence rate across age groups, our country’s trend is the same as other countries, with the incidence evidently increasing with age. Clearly, we should continue treating the elderly as a key target group for epidemic prevention. For example, places where the elderly frequent, such as hospitals, nursing homes, commercial establishments and so on, are all places where disease prevention efforts should be strengthened. At present, our country's nosocomial infection control is governed by the "Guidelines and standard operating procedures for hospital environmental examination of Legionnaires' disease bacteria and related measures" and associated rules. Also, commercial establishments should refer to the "Business sanitation standards" issued by county governments or the "Business sanitation self-regulation act" promulgated by local governments. The commercial establishments should be encouraged to follow the spirit of self-regulation to strengthen the cleaning and disinfection of their water supply systems, central air-conditioning’s cooling towers and other water facilities.

In terms of the monthly distribution of cases, the number of cases reaches its peak in summer. This result is consistent with other countries, but our curve is not as obvious as other countries. This might be due to our country’s climate, as our temperature does not change as much across seasons as other countries. Therefore, in terms of the goal of promoting self-regulation among commercial establishments, the operators of these establishments should strengthen self-regulation in normal days and make sure that their watering supply systems and water-cooling towers are sanitized, especially those water systems in commercial establishments frequented by the elderly and people with a poor immune system. This would help keep people away from the harm of Legionnaires' disease and protect the health of the citizens.

References

1. TCDC. Communicable Disease Control Workbook. Available at : http://www2.cdc.gov.tw /ct.asp?xItem= 6469&ctNode=1733&mp=1.
2. TCDC. Guidelines for prevention on suspected Legionnaires' disease cluster events in public places. Available at:http://www.cdc.gov.tw/diseaseinfo.aspx?treeid=8d54c504e820735b&nowtreeid=dec84a2f0c6fac5b&tid=16CD29A279936655.
3. TCDC. The Notes on Environmental specimens sampling of Legionnaires' disease. Available at : http://www.cdc.gov.tw/diseaseinfo.aspx?treeid=8d54c504e820735b&nowtreeid=dec84a2f0c6fac5b&tid=16CD29A279936655.
4. TCDC. Definition on communicable disease and matters on Epidemic prevention sampling and delivery. Available at : http://www.cdc.gov.tw/lp.asp?ctNode=2415&CtUnit=1404&BaseDSD=7&mp=1.
5. Lam MC, Ang LW, Tan AL et al. Epidemiology and Control of Legionellosis, Singapore. Emerg Infect Dis 2011;17:1209-15.

6. CDC. Legionellosis—United States, 2000–2009. MMWR 2011;60:1083-6.
7. ECDC. Annual Epidemiological Report 2011- Reporting on 2009 surveillance data and 2010 epidemic intelligence data - Legionnaires' disease（Legionellosis）Surveillance Report 2011:30-2.

Diagnosis of Legionnaires' Disease and Detection of *Legionella* from Environment

Yu-Lan Wang, Li-Rong Cheng, Jei-Kai Tan, Chuen-Sheue Chiang

Research and Diagnostic Center, Centers for Disease Control, Taiwan

Abstract

Legionnaires' disease is a life-threatening lung infection commonly seen in patients of community-acquired and hospital-acquired pneumonia. The key methods in determination of Legionnaires' disease are to conduct appropriate microbiological diagnosis. However, there is no single laboratory method that being sensitive, specific, and rapid enough, simultaneously. Therefore, most of the laboratories conducting testing for Legionnaires' disease usually employ multiple methods for multiple specimens from a single patient and make decision about the choice of the methods on the basis of the epidemiological characteristics of Legionnaires' disease in their regions, and the understanding of the characters and limitations of the methods. The testing methods and the diagnostic criteria for confirmation of Legionnaires' disease currently used are mostly the same among countries in the world, except that the diagnostic criteria for serological test are different among them. The annual number of reported Legionnaires' disease cases in Taiwan ranged from 550 to 1,700 during 2000 and 2012 with 40 to110 confirmed cases. The average male to female ratio of confirmed cases was 2.9. The 70 years and older age group recorded the highest number of confirmed cases. An average of 68% of confirmed cases were determined by urinary antigen test and 25% by serological test. The strains of *Legionella* spp. were isolated from the specimens of 36% of confirmed cases. The positive rates for PCR assay were higher than that for culture. The PFGE pattern analysis indicates that 22 strains isolated from clinical specimens of confirmed cases have the same genotype as those from the corresponding environmental specimens since the PFGE assay was applied to the investigation of environmental sources in 2005. Owing to the difficulties in laboratory tests, the number of Legionnaires' disease cases diagnosed has probably been much less than that really occurred. To be able to rapidly and correctly diagnose a patient with Legionnaires' disease is a key step in reducing the severity and mortality of the disease. Therefore, the future development in diagnosis of Legionnaires' disease will focus on the development and

application of rapid test technologies, and the strategies for performing laboratory test would be to apply multiple tests simultaneously with multiple specimens from a single case to elevate the sensitivity of the laboratory diagnostic tests.

Keywords: Legionnaires' disease, *Legionella pneumophila*, examination for environmental specimens, pneumonia, microbiological diagnosis

Introduction

Legionnaires' disease is a serious, life-threatening lung infection and also a major cause commonly seen in patients of community-acquired and hospital-acquired pneumonia. The Legionnaires' disease is caused by infection with Legionella bacteria which also have been found to cause a less serious and self-limiting disease called Pontiac fever. Legionnaires' disease and Pontiac fever are the two most common types of legionellosis. Legionnaires' disease was first recognized during a large-scale outbreak of pneumonia of unknown origin occurred among people attending American Legion convention in Philadelphia, USA, in 1976. A new pathogen was isolated from lung autopsy specimens collected from cases dying from Legionnaires' disease, which was later named *Legionella pneumophila*. To date, over 50 species comprising 70 serotypes of Legionellae have been identified and the number of the species and serotypes is increasing continuously. Among these species, 25 were found to be associated with human diseases [1-2]. The species *Legionella pneumophila* includes at least 16 serotypes that are responsible for more than 90% of cases of Legionnaires' disease. Of these, serotype 1 is the most important type since 85% of Legionnaires' disease cases were caused by it [2-4].

The Legionellae are anaerobic, non-spore forming, gram-negative, rod-shaped bacteria. The conditions and nutritional requirements for in vitro culture of Legionellae are very complex. The characteristic of being different from other bacteria is that Legionellae are able to reproduce within macrophages. Legionella species survive widely and commonly in natural environment, including rivers, ponds, lakes, and soils, can live in moist environments for a long time, and can multiply in natural environment with conditions at temperature from 0 to 68 ℃ and pH-value from 5.0 to 8.5. They obtain nutrients necessary for reproduction from symbiotic microorganisms and the nutrient content existing in the water system and often use free-living amoebae in environments as a natural host for survival and growth. Legionellae are tolerant to chlorine in the water and can survive in biofilms. These characteristics have promoted its tolerance to microbial biocides and chlorine dioxide disinfection. The sources of infection for most cases of Legionnaires' disease have been traced to artificial aquatic environments. When the number of Legionella bacteria in aquatic environment reaches to a certain level, they can be spread to susceptible people through aerosols and cause infection and illness. However, the association between the number of bacteria in water system and the risk of infection is an issue that still has not been clarified to date [5]. Although infections of wound through contact of contaminated water have occurred in very rare cases, Legionnaires' disease is not transmitted from person to person through physical contact.

The occurrence of Legionnaires' disease case in Taiwan was first documented in 1985 [6]. In 1989, the National Institute of Preventive Medicine (one of the antecedent agencies of the Taiwan Centers for Disease Control, Taiwan CDC) introduced testing technologies for *Legionella spp.* from the USA and Japan and, in 1993, started to receive notification of Legionnaires' disease cases and conducted the testing of specimens from these cases. In addition, a large-scale environmental investigation on Legionella bacteria was performed in this country during this period. Later, Legionnaires' disease was added to the list of Category 3 communicable diseases when the Communicable Disease Control Act was revised in 1999. At the early stage when Taiwan CDC was established (in July 1999), the testing of specimens from cases of infectious diseases were conducted by laboratories settled in the Headquarter and Branches of Taiwan CDC. In this stage, the diagnosis of communicable disease cases were made based on serological testing results in most of the laboratories except that in the Headquarter. Moreover, there are no unified reference values for serological test in determination of positive cases. Starting in 2004, three different specimens (respiratory tract secretions, urine, and serum) required for the diagnosis of diseases should be collected simultaneously from all cases when they are notified. The laboratory testing for specimens of all the notified cases from around the country were conducted by laboratory at Taiwan CDC since 2005. In addition, it was required that the environmental specimens related to the positive cases should be collected and tested since 2004, and the comparison of the strains isolated from clinical specimens with those from environmental specimens of positive cases should be made starting in 2005. The policy of investigation and analysis of environmental infection sources were, therefore, formally initiated.

Current status and international development of diagnosis for Legionnaires' disease

The major clinical presentation of Legionnaires' disease is pneumonia although non-lung forms of infection and symptoms may occur in a small number of immune dysfunction cases. The manifestations of pneumonia caused by Legionellae are not specific in terms of the clinical signs, physical examination findings, and chest x-ray results, i.e., they cannot be clearly distinguished with those caused by infections with other microorganism. Therefore, the key methods in diagnosis of Legionnaires' disease currently are to conduct appropriate microbiological diagnostic testing. The methods of laboratory testing for diagnosis of Legionnaires' disease now used internationally include direct fluorescent antibody (DFA) test, culture, urinary antigen tests, serological test, and nucleic acid detection. The characteristics and limitations for each of these methods are shown in Table 1. The genetic typing methods are mainly used for the purposes of determining the environmental infective sources involving human infections so that the outbreak could be effectively controlled and prevented. Although the Legionnaires' disease has undergone several years of development since it was identified, there still has no single laboratory method that being sensitive, specific, rapid, and timely enough, simultaneously. Therefore, in order to effectively perform diagnostic testing for patients of Legionnaires' disease, laboratories in international community usually make decision about what testing methods should be adopted on the characteristic epidemiology of Legionnaires' disease in their regions, and the understanding of the characteristics and limitations of the methods.

Table 1. The characteristics and limitations of clinical diagnosis for Legionnaires'disease

Tests	Sensitivity/specificity (%)	Time for testing	Limitations
Direct fluorescent antibody staining	25-70 / 95	2-4 hours	Sensitivity may change with type of specimen. A false-positive result may occur. The testing results may be affected by skills and experience on the tests.
Culture	＜10-80 / 100	3-7 days	The quality of sputum specimens is difficult to be controlled. The amount of bacteria in sputum varies with disease progression. The testing results may be affected by skills and experience on the tests.
Urinary antigen test[1]	70-90 / ＞99	15 min-3 hours	This test is used only for *Legionella pneumophila* serogroup type 1 strain.
Serological test	60-80 / ＞95	1-10 weeks	The results based on four-fold increase in antibody titer is just used for retrospective diagnosis that provides only a small benefit for patient treatment in early stage of the disease.
Nucleic acid detection[2]	80-100 / ＞90	Within 4 hours	This may produce unclarified false-positive results.

1 The similar method was developed for applying to non-urinary specimen although its efficiency needs to be evaluated and recognized. However, no commercial kits for detection of urinary antigen from *Legionella pneumophila* non-serogroup type 1 strain have been developed.

2 The data provided here is appropriate for respiratory tract specimen only. Although this method is also applied to urine and serum specimen, the sensitivity is ranged from 30-80%.

Although the direct fluorescent antibody staining can be performed immediately after receiving clinical specimens, including tissues and respiratory tract excretions, the inconsistent sensitivity and cross reactions with other microorganisms will influence the specificity. Therefore, the diagnosis of Legionnaires' disease cannot be made merely based on the positive results from the direct fluorescent antibody staining, which is usually made under supports of results from other tests. The culture analysis has a specificity of 100% and it has been considered as the gold standard in diagnosis of Legionnaires' disease up to now. The number of Legionella strains that can be determined through culture is the largest as compared with other tests, and culture method can be employed for various types of specimens although excretions of lower respiratory tract such as sputum and bronchial materials are more valid. The major limitations of culture method depend on the validity of the specimens. The isolation rates for specimens from severe pneumonia patients are higher (＞90%), and only 15-25% from mild cases [7]. In addition, the fact that less than a half of Legionnaires' disease patients excrete sputum, the quality of the clinical sputum specimens, and the survival rate of Legionella bacteria in the excretions will also influence isolation rate. Except experience on operation of culture method, a better isolation rate may also be obtained when the culture is performed under the premise of specifically focusing on or having a high suspicion for Legionnaires'diseases [8]. The urinary antigen test was a

breakthrough development in the diagnosis of Legionnaires' disease and has become the method that is currently used most frequently in clinical practice. Because of the clinical application of the method, the number of confirmed Legionnaires' disease cases has significantly increased by several folds in countries worldwide [9-11]. Several advantages have been described about the urinary antigen test. For example, it has ideal sensitivity and specificity and can be used as a tool for early detection since the antigen is detectable one day after the date of onset and continually maintains from several days to several weeks, and the results of the testing will not be influenced even though the patients have received antibiotic treatments. Therefore, it has been an effective and useful tool in conducting epidemiological investigation. The methods currently used for urinary antigen test mainly include enzyme immunoassay (EIA) and rapid immunochromatographic assay. In concentrated urine specimens, the sensitivity of the tests can be increased significantly [8]. However, since the concentration process is time-consuming and tedious, it usually applied only for very small number of problematic cases but not for general cases in routine practices. In serological tests, the serum conversion, a four-fold increase in antibody titer, is considered as the criteria with a higher credibility for laboratory diagnosis of Legionnaires' disease in serological test [12]. However, this method has the disadvantages of being unable to differentiate strains and serotyping and poses the cross-reactivity problems. The main techniques for serological test include indirect fluorescent antibody (IFA) staining and EIA, both of them are considered as the standard procedures. Because of the characteristics of evaluation criteria, accuracy, and automated operation, the EIA techniques have been adopted by more and more laboratories.

As the nucleic acid technique was developed, the choices of methods for detection of Legionella bacteria were much more diverse. Moreover, some commercial kits have been created and used for rapid tests, such as quantitative real-time polymerase chain reaction (PCR) tests and isothermal loop amplification (LAMP) assays. Also, many laboratories have designed their own analysis procedures and applied them to routine testing. For specimens from lower respiratory tract, the nucleic acid test has the sensitivity equal to or even higher than that of culture, can overcome the conditions arising from poor quality of specimens for culture analysis, can be used for testing of specimens collected from patients in the prodromal stage, and even can be applied to other specimens, such as urine, serum, and white blood cells. Therefore, to perform testing simultaneously for multiple specimens from a single patient could effectively increase its sensitivity [13]. The methods currently used for genetic typing of Legionella bacteria include amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), restriction endonuclease analysis (REA), and arbitrarily primed PCR. The European Working Group for Legionella infections (EWGLI) mainly apply the methods of AFLP and MLST while the US CDC uses the PFGE for typing of Legionella species more frequently.

Diagnosis of Legionnaires' disease and detection of *Legionella* from environment in selected countries

The methods of microbiological diagnostic testing currently adopted by selected countries and the criteria for diagnosis of Legionnaires' disease are shown in Table 2. There is a slight difference in testing methods and the diagnostic criteria for confirmation of Legionnaires' disease among the countries. Culture (for specimens from respiratory tract excretions, lung tissues, pleural fluids, and other sterile parts of the body) and urinary antigen test are commonly applied as the methods for confirmation of Legionnaires' disease cases, but the diagnostic criteria for serological test are different among the countries. The common criterion for laboratory diagnosis of Legionnaires' disease cases among the countries is that a case will be considered as a confirmed case as long as a positive result is obtained from either one of the tests. For surveillance or investigation of Legionella bacteria in environment, either qualitative or quantitative analysis is performed depending on the purposes of the tests and the extents to which the tests are expecting to reach. The method frequently used in environmental surveillance for Legionella bacteria is culture analysis although nucleic acid tests are also employed in a small number of conditions. In addition, except the definitions of cases of community-acquired and hospital-acquired Legionnaires' disease, the United States of America, Europe, and United Kingdom defined the cases of travel-associated Legionnaires' disease as those who have stayed overnight away from their own homes for 2-10 days before onset of illness.

Table 2. Laboratory methods and criteria for diagnosis of Legionnaires' disease in selected countries

Tests	USACDC	WHO	Europe ECDC/ ELDSNet	UK HPA	Ireland NDSC	Australia NSW	Hong Kong	Singapore
Culture	C[3]	MT[3]	C	C	C	C	C	C
Urinary antigen test	C	MT	C	C	C	C	C	C
Serological test[1] ≧4-fold increase, SG1	C	OT[3]	C	C	C	C	C	C
≧4-fold increase,non-SG1	S[3]	OT	S	N	C	C	C	C
Single high antibody titer	N[3]	N	S	S	S	S	N	S
DFA staining[2]	S	OT	S	S	S	S	N	C
Nucleic acid test	S	N	S	N	N	S	N	N

[1] The criteria include three parts: four-fold increase of antibody titer against *Legionella pneumophila* serogroup 1 (SGI), four-fold increase of antibody titer against *Legionella pneumophila* non-serogroup 1 (non-SGI), and high antibody titer in a single serum sample. The definition of single high antibody titer varied in different countries, including >1:64, >1:128, or >1:1024.

[2] This includes other techniques similar to the method of directly detecting agent in specimens and to that of identifying pathogens by staining.

[3] C means that the positive cases were considered as confirmed cases, and S means that the positive cases were considered as suspected cases. N means that the methods were not employed by the country. MT and OT represent mandatory testing and optional testing, respectively.

Generally, the testing of specimens for Legionella bacteria from clinical cases was performed by clinical laboratories or local health authorities, then the results were reported to central health authorities, the respective responsible agencies are Centers for Disease Control (CDC) in US, Health Protection Agency (HPA) in UK, and National Institute of Infectious Disease (NIID) in Japan. The strains isolated might also be sent to central health authorities for further analysis. The HPA sometimes conducted testing for specimens commissioned by others but it is charged. In Hong Kong, the Public Health Laboratory Services Branch, Center for Health Protection, conducted testing for clinical specimens. As to the testing of environmental specimens for monitoring of Legionella bacteria, the specimens were tested by laboratories accredited by the United Kingdom Accreditation Service (UKAS) in UK, by the National Association of Testing Authorities (NATA) to ISO 17025 in Australia, and by the authorized organization to ISO 13485 and 9001:2000 in Germany. In Ireland, the testing was conducted by laboratories that have received external evaluation and certificated. In the USA, the testing was performed by certain laboratories.

Diagnosis of Legionnaires' disease and detection of *Legionella* from environment in Taiwan

The methods currently used by the laboratory in Taiwan CDC for detection of Legionella bacteria from clinical specimens include culture, urinary antigen tests, and serological tests. For the culture analysis, the specimens for isolation of Legionella species are taken from lung tissue, respiratory tract excretions, pleural fluids, blood, or other sterile parts of the body. A total of 25 species of Legionellae can be recognized by the laboratory currently. As for urinary antigen tests, the EIA assay is employed for detecting the antigen of *Legionella pneumophila* serogroup type 1 strain. As for serological tests, the IFA staining method is performed for antibody in the serum collected during the recovery stage (three to eight weeks after onset of illness) and acute stage. A four-fold increase in antibody titer to $\geqq$1:128 against *Legionella pneumophila* is considered as positive. Currently, the antibody against *Legionella pneumophila* serogroup type 1 to 6 strains can be detected through this method. Any case with a positive result in either one of the three tests is defined as a confirmed case. In addition, multiplex quantitative real-time PCR assays is also employed for testing specimens which used in culture analysis for detection of both *Legionella pneumophila* strains and all other Legionella strains simultaneously.

The environmental specimens undergoing testing for Legionella bacteria were limited to those collected from the environments where the confirmed cases were staying at or having a contact with during the incubation period for Legionnaires' disease. The environments probably include the houses or working places of the cases, and the recreation resorts or facilities, hotels, or hospitals where the cases have stayed. The specimens might be collected from faucets, shower faucets, drinking machine, and water-cooling tower. Since the testing of environmental specimens was intended to identify sources associated with the human cases,

the qualitative analysis method was used for the isolation and determination of the strains. Once the strains were isolated from the specimens collected from both the confirmed cases and the environments associated with the cases and belong to the same serogroup, the PFGE pattern analysis was carried out for comparison of genome types of the strains so that the relationship between the cases and the environments could be clarified. Moreover, the MLST analysis was unperiodically performed for strains isolated from both clinical and environmental specimens, and the international epidemiological information was collected for comparison of the types causing epidemics in other countries.

Other laboratories intending to perform testing for Legionella bacteria in Taiwan will have to file an application to the Department of Health and get certificates in recognition of meeting the eligibility criteria for conducting testing for infectious diseases. The recognition criteria include that the laboratories shall have the abilities of conducting the above-mentioned three methods of analysis: culture, urinary antigen tests, and serological tests, and continually participate in the proficiency testing program for general microbiological diagnostic testing. The institutes currently meet the criteria are the Kaohsiung Veterans General Hospital and the Super Laboratory Co., Ltd. In addition, the institutes intending to conduct testing for Legionellae in water samples must meet the criteria that the laboratories shall have the ability of performing quantitative analysis of environmental water samples, and continually participate in the proficiency testing program for general microbiological diagnostic testing. Currently, eight hospitals meet the criteria. These are the Kuo General Hospital, Kaohsiung Veterans General Hospital, Kaohsiung Chang Gung Memorial Hospital, China Medical University Hospital, Chung Shan Medical University Hospital, Chiayi Chang Gung Memorial Hospital, Keelung Chang Gung Memorial Hospital, and Chang Gung Memorial Hospital at Linkou. Laboratories accredited by the Taiwan Accreditation Foundation (TAF) in compliance with the requirements of ISO 17025 are also eligible for testing for Legionella bacteria. Five laboratories now are recognized. These are the Super Laboratory Co., Ltd., Societe Generale de Surveillance (SGS) Group in Taiwan, Blue-Formosa Environmental Technology Corporation, Food Industry Research and Development Institute, and Siuding Laboratory Technology Co., Ltd.

The annual number of reported Legionnaires' disease cases in Taiwan ranged from 550 to 1,700 during 2000 and 2012 and that of confirmed cases ranged from 40 to110. The average male to female ratio of confirmed cases was 2.9. The 70 years and older age group has always recorded the highest number of confirmed cases, followed by the age group 60-69 years old and 50-59 years old. The number of confirmed cases aged 50-59 years old appeared an increasing trend during the period between 2005 and 2010. The pie charts in the Figure 1-2 show the percentage distribution for strains of Legionellae isolated in Taiwan during 2002-2011. The chart in the Figure 1 represents the strains that have caused the infections of Legionnaires' disease and the chart in the Figure 2 displays the strains isolated from specimens collected form environment associated with the confirmed cases of Legionnaires' disease.

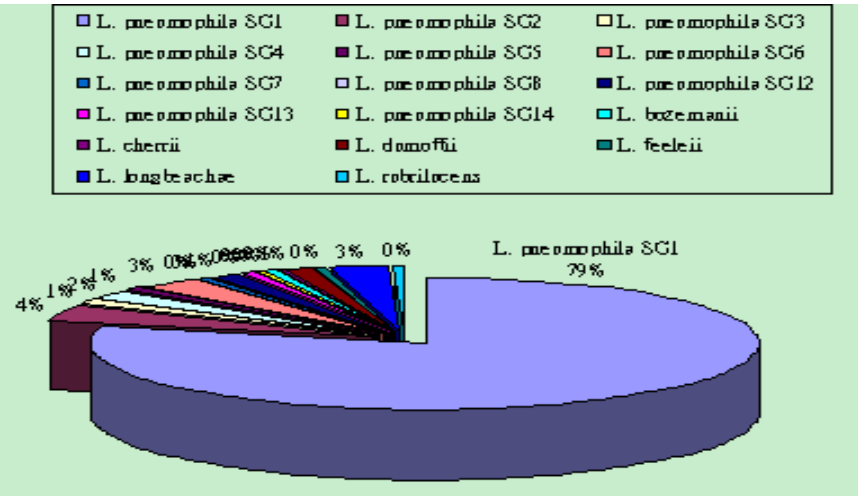

Figure 1. Pie chart of percentage distribution of *Legionella* clinical isolates (211 strains) in Taiwan during 2002-2011

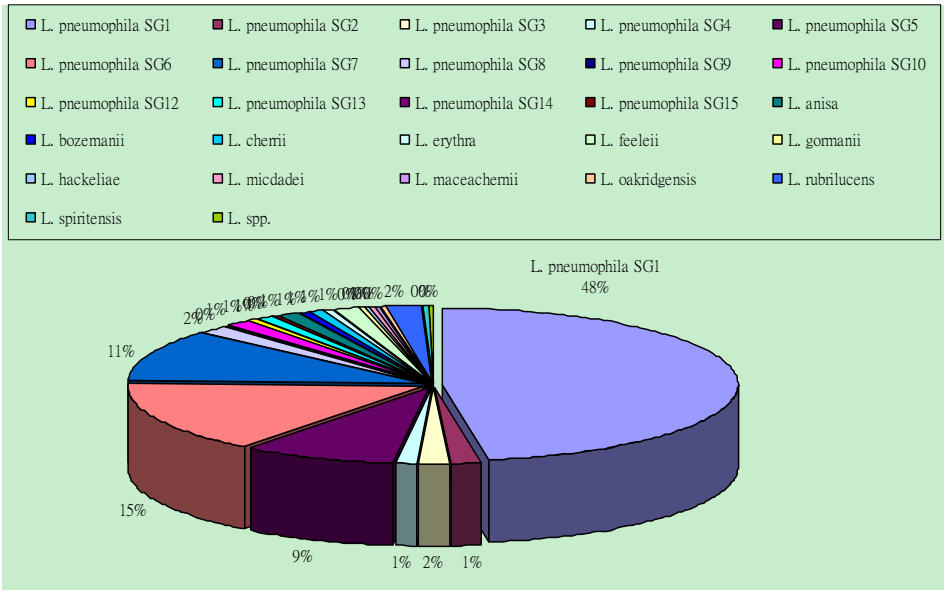

Figure 2. Pie chart of percentage distribution of *Legionella* enviromental isolates (548 strains) in Taiwan during 2002-2011

Table 3. Detection results by different laboratory methods and environmental specimens during 2006-2011

Years	No. of confirmed cases	Percentage of confirmed cases U/C/S (%) [1]	Positive rate of confirmed cases U/C/S (%) [2]	PCR positive rate Lp/Lspp (%) [3]	Positive rate for environmental specimens (%)	No. of clinical strains with the same PFGE genotype as environmental strains (serotype) [4]
2006	54	63/11/26	63/26/72	NA	15.7	2 (SG1)
2007	56	61/10/29	61/37/81	NA	12.2	2 (SG1, SG3)
2008	69	62/05/33	62/30/97	NA	11.3	4 (SG1)
2009	84	76/06/18	76/40/70	NA	15.3	3 (SG1)
2010	102	71/08/21	71/45/60	53/64	12.7	6 (SG1, SG2, SG5)
2011	97	67/06/27	67/33/61	43/46	9.9	3 (SG1)
Total	462	68/07/25	68/36/71	48/55	12.6	20

1The number of positive results was added by one only for the priority test method although the positive results may also be obtained by other test methods. The priority order for calculating the positive results is urinary antigen test (U), culture (C), and serological test (S). The sum of the percentage for the three methods is one hundred.

2 The number of positive results for one test method was added by one whenever a positive result was obtained by this method.

3 In 2010, the PCR assay was first applied to the specimens same as those used for culture for detection of Legionella species. Lp: *Legionella pneumophila*, Lspp: all Legionella species

4 All 20 isolates belong to the strains of *Legionella pneumophila*. The words within parentheses represent the serogroups of the strains identified in each of the years. The PFGE pattern analysis was first conducted in 2005 and 2 clinical strains with the same genotype as environmental strains were identified in the same year.

Table 3 shows the analysis on testing of clinical specimens from notified Legionnaires' disease cases by different laboratory methods and of environmental specimens related to the notified cases between the years of 2006, when the government required that all specimens from notified Legionnaires' disease cases be conducted by the laboratory in the Taiwan CDC , and 2011. In Table 3, the percentage of confirmed cases means the number of confirmed cases identified by cultures, urinary antigen tests, or serological tests among the all confirmed cases while one of the three methods was chosen as the priority method, and the positive rate of notified cases means the number of positive cases among all the notified cases tested by each of the three methods performed by laboratory in the Taiwan CDC. As shown in Table 3, an average of 68% of confirmed cases were determined by urinary antigen test and 25% by serological test. The strains of Legionellae were isolated from the specimens of 36% of confirmed cases. The serological tests were positive for an average of 71% of notified Legionnaires' disease cases. The positive rates for PCR assay were higher than that for culture. The PFGE pattern analysis indicates that 20 strains isolated from clinical specimens have the same genotype as those from the corresponding environmental specimens that seven of them were collected from sites in hospitals, 4 in resorts and hotels, and 9 at homes.

The testing results for Legionella bacteria in specimens collected from environments related to the confirmed cases are shown in Table 4. The qualitative testing found that the positive rate for specimens collected from home and working environments was less than 20% while those from water towers, faucets or shower heads in medical facilities and those from pond (well) water, hot water springs, faucets, and cooling towers in resort facilities was more than 20%.

Table 4. Detection of *Legionella* from environmental specimens related to the confirmed cases during 2002-2011

Homes and working sites		No. of specimens	No. of positive specimens	Positive rate (%)
Bathroom	Faucets	945	97	10.3
	Shower heads	474	49	10.3
	Toilets	15	1	6.7
	Others	3	0	0.0
Inside home	Kitchen faucets	728	68	9.3
	Drinking water	340	43	12.6
	Others	23	1	4.3
Outside home	Pond (well) wate	32	2	6.3
	faucets	188	21	11.2
	Water towers	184	9	4.9
	Cooling towers	65	4	6.2
	Others	13	1	7.7
Other unknown environments		80	6	7.5

Medical facilities	No. of specimens	No. of positive specimens	Positive rate (%)
Faucets	327	98	30.0
Shower heads	111	28	25.2
Drinking water	76	1	1.3
Water towers	22	8	36.4
Cooling towers	28	4	14.3
Others	24	0	0.0
Resort facilities			
Faucets	110	25	22.7
Shower heads	160	18	11.3
Toilets	9	0	0.0
Drinking water	26	2	7.7
Pond (well) water	3	1	33.3
Water towers	11	1	9.1
Cooling towers	23	5	21.7
Hot water spring	80	20	25.0
Swimming pools	18	1	5.6
Others	15	0	0.0

The number of Legionnaires' disease cases diagnosed has been much less than that really occurred for a number of reasons. For example, the symptoms and signs of Legionnaires' disease are not specific for making an early diagnosis, the patients with pneumonia will not necessarily undergo testing for Legionella species in clinical diagnosis, the tests that most hospitals are capable of conducting usually do not cover the test for Legionella, and various limitations in existing laboratory tests. In fact, to be able to rapidly and correctly diagnose a patient with Legionnaires' disease is a key step in reducing the severity and mortality of the disease [14]. Therefore, the future development in diagnosis of Legionnaires' disease will focus on the applications of rapid test technologies. Although the commercial kits for *Legionella pneumophila* non-serogroup 1 urinary antigen might be still unavailable because of the consideration of market demands, nucleic acid test could be the method that has the opportunity to be further developed and applied. The future strategies for performing laboratory testing in infectious disease control would be to apply multiple test methods simultaneously to multiple specimens from a single case to maximize the effectiveness of the laboratory diagnostic practices. Moreover, the feasibility and effectiveness of applying PCR assay to the routine diagnostic testing should be continually evaluated in order to determine to what extent the PCR assay could be applied based on the evaluation. In addition, due to the characteristics of Legionella bacteria that contain numerous and diversified genotypes, the PFGE analysis is inappropriate for comparison of the genotypes in this country with those epidemic in foreign countries or comparison of genotypes between different regions. However, since the PFGE analysis is useful in the investigation of infection sources, it will be used continually in the investigation into the environmental sources blamed for causing human infections. While conducting the investigation of the infection sources causing the infections among travelers, the MLST analysis can be used for comparison of genotypes between regions or countries.

References

1. Gobin I, Newton PR, Hartland EL, et al. Infections caused by non pneumophila species of *Legionella*. Rev Med Microbiol 2009;20:1-11.
2. Fields BS, Benson RF, Besser RE. *Legionella* and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev 2002;15:506-26.
3. Muder RP, Yu VL. Infection due to *Legionella* species other than *L. pneumophila*. Clin Infect Dis 2002;35:990-8.
4. Yu VL, Plouffe JF, Pastoris MC, et al. Distribution of *Legionella* spcies and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. J Infect Dis 2002;186:127-8.
5. O'Neill E, Humphreys H. Surveillance of hospital water and primary prevention of nosocomial legionellosis: what is the evidence? J Hosp Infect 2005;59:273-9.

6. Liu YC, Cheng DL, Wang JH, et al. Legionnaires' disease: a case report. J Formos Med Assoc 1985;84:1180-5.
7. Murdoch DR. Diagnosis of *Legionella* infection. Clin Infect Dis 2003;36:64-9.
8. Diederen BMW. *Legionella* spp. and Legionnaires' disease. J Infect 2008;56:1-12.
9. Joseph JA. European working group for *Legionella* infections. Legionnaires' disease in Europe 2000-2002. Epidemiol Infect 2004;132:417-24.
10. Formica N, Yates M, Beers M, et al. The impact of diagnosis by *Legionella* urinary antigen test on the epidemiology and outcomes of legionnaires' disease. Epidemiol Infect 2001;127:275-80.
11. Joseph CA, Ricketts KD. Legionnaires' disease in Europe 1995-2004: a ten year review. In: Cianciotto NP, Kwaik Y, Edelstein PS, Fields BS, Geary DF, Harrison TG, et al., Eds. *Legionella*: State of the art 30 years after its recognition. Washington, D.C.: American Society for Microbiology,2006;89-93.
12. Plouffe JF, File TM, Breiman RF, et al. Reevaluation of the definition of legionnaires' disease: use of the urinary antigen assay. Clin Infect Dis 1995;20:1286-91.
13. Murdoch DR, Walford EJ, Jennings LC, et al. Use of the polymerase chain reaction to detect *Legionella* DNA in urine and serum samples from patients with pneumonia. Clin Infect Dis 1996;23:475-80.
14. Benin AL, Benson RF, Besser RE. Trends in legionnaires' disease, 1980-1998: declining mortality and new patterns of diagnosis. Clin Infect Dis 2002;35:1039-46.

The Taiwan Epidemiology Bulletin series of publications is published by Centers for Disease Control, Department of Health, Taiwan (R.O.C.) since Dec 15, 1984.
Publisher : Feng-Yee Chang
Editor-in-Chief : Yi-Chun Wu **Telephone No :** (02) 2395-9825
Executive Editor : Li-Gin Wu, Hsiu-Lan Liu **Website :** http://teb.cdc.gov.tw/
Address : No.6, Linshen S. Road, Taipei, Taiwan 100 (R.O.C.)
Suggested Citation :
[Author].[Article title].Taiwan Epidemiol Bull 2012;28:[inclusive page numbers].